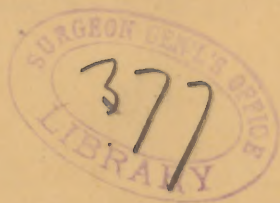


Bertolet. (R. M.)

on the guaiacum process + +





## ON THE GUAIAIACUM PROCESS

FOR THE

DETECTION OF BLOOD AS A VALUABLE AID IN DISTINGUISHING  
NUCLEATED FROM NON-NUCLEATED RED BLOOD DISKS.

By R. M. BERTOLET, M.D.,

MICROSCOPIST TO THE PHILADELPHIA HOSPITAL.



THE large size, elliptical shape, and the central oval nucleus, characterizing the red blood disks of birds, fishes, and reptiles, are generally considered as ample land-marks to enable even the merest novice in microscopy to distinguish the blood of any animal belonging to any one of these three great classes from the flat, biconcave, non-nucleated disks characterizing human and mammalian blood. Such is the case, at least, as far as fresh specimens are concerned, but it is far from an easy matter to discriminate between these two kinds of blood when they have once become clotted and thoroughly dried. What great changes this process of drying implies! The corpuscles are no longer flat and smooth, but shrunken and crenated; their outlines are no longer regular and sharply defined, but irregular, jagged, or stellate; they are so intimately agglutinated with one another, and hidden in the meshes of the coagulated fibrin, that it becomes difficult, even in thin fragments, to recognize them.

When blood is very thinly spread out and rapidly dried upon a glass slide, then the disks remain firmly stretched and the diameters are not materially altered;<sup>1</sup> but when left to dry in any other manner, the great shrinkage and other alterations in the corpuscles just enumerated take place. Under these circumstances it becomes an extremely difficult task

<sup>1</sup> Vide Stricker, Gewebe Lehre, Leipzig, 1871, p. 280.



to decide which of the two kinds of blood we have to deal with. The size and shape of the dried corpuscles are not to be depended upon; to resuscitate them exactly to their original condition by the imbibition of fluids is an impossibility, especially so when irrigation with pure water is resorted to, as recommended in *Wharton and Stillé's Medical Jurisprudence*, § 758, 3d ed., 1873. Distilled water not only completely abstracts the colouring matter from the red corpuscles, so that the author of the article on "Microscopic Evidence" found it necessary to render them "more obvious" by adding iodine or red aniline solutions, but it also finally causes both the colourless and coloured disks to disintegrate and eventually to disappear.<sup>1</sup> These are sufficient reasons to condemn the use of water in the examination of dried blood. There are other less objectionable fluids that may be employed—the sulphate of soda in solution is excellent; I have found none, however, so admirably adapted as slightly acidulated pure glycerine. It does not evaporate, so that specimens mounted in it can be kept an indefinite time for study. Its slight acidity will render the nuclei more distinctly visible when present.

How rarely, though it must occur in medico-legal cases, that the microscopic expert has given to him blood that has not already been more or less completely dried. In very many of these cases it is of the utmost importance to determine which of the two kinds of blood it is, and the determination of the presence or absence of the nuclei in the red corpuscles is of more importance than all the other features possessed by the different bloods, frequently virtually deciding the guilt or innocence of the accused.

The addition of serum-like and other liquids to small fragments of the questionable blood, mounted beneath the microscope, is not to be neglected, yet very unsatisfactory, for as yet there is no liquid known which will precisely restore the size and shape of the once dried corpuscles. Those from the mammalian animals can be made to swell out, but become circular in outline instead of biconcave as seen in the fresh state; while the elliptical disks become more nearly round and their outlines pale and indistinct. Hence the great importance attached to the nucleus, for it frequently is the only distinctive mark between the two kinds of blood. In cases where the blood stains are alleged to be those of some animal having the nucleated disks, it behooves the examiner to use every exertion to develop, if possible, the nucleus.

Although few experienced microscopists would hesitate in pronouncing upon the existence or non-existence of a nucleus in any given corpuscle, it is an extremely difficult matter to establish the fact in the minds of the jury, and still more difficult to demonstrate it clearly to those who are unaccustomed to making microscopic observations.

<sup>1</sup> Dr. Kline, in *Burdon-Sanderson's Handbook for the Physiological Laboratory* 1873, p. 29.

Any method, therefore, which will enable the examiner to demonstrate without the shadow of a doubt, not only in his own mind but also to a jury, the presence or absence of the nucleus cannot fail to be accepted as a most valuable corroborative proof. Such a method for differentiating between the two kinds of blood, I believe is to be found in the well-known guaiacum test for detecting blood stains.

If a microscopic preparation, mounted in feebly acidulated glycerine or other suitable fluid, is carefully irrigated with a properly prepared alcoholic solution of guaiacum resin; then when a very small quantity of the ethereal solution of the peroxide of hydrogen (ozonic ether) is introduced beneath the glass cover, a bluing, varying from a light sapphire to a deep indigo shade, will be observed in the several corpuscles of mammalian blood. Though the degree of coloration of the different corpuscles presents considerable variety, yet all parts of the individual corpuscle itself are coloured uniformly. If the blood has been derived from an animal having *nucleated corpuscles*, an *entirely different* picture is presented; when treated by these same reagents, *the nucleus is seen as a sharply defined, dark blue body, while the protoplasm surrounding it assumes a more delicate violet hue.* Sometimes the other parts of the blood-globules are not the least coloured; in all instances, however, the nucleus is deeply stained and easily recognizable when present, even by the uneducated eye.

The charming picture, that can thus be produced by differentiating the nucleus from the other parts of the corpuscles, may be of invaluable service where it is desirable to *convince* the jury by optical proof of the existence of the nucleated blood-globules. Furthermore, in those cases where the blood is alleged to pertain to an animal having the nucleated disks and found not to be such by the ordinary methods of investigation, I believe it to be the duty of the expert to employ this method of *searching* for the nucleus, as stated in my testimony at the trial of Talifero, at Martinsburg.<sup>1</sup>

The attention of the profession has not yet, as far as I can ascertain, been called to this, the ultimate application of the guaiacum process for the detection of blood. The original papers of Dr. Day, of Geelong, Australia, who first established the reliability of the guaiacum process as a blood test, have been inaccessible to me, yet Dr. Alfred S. Taylor, to whom they were referred, and who has himself investigated with this process, states:—

“It simply enables the operator to say whether that which he is examining is the colouring matter of a red-blooded animal. It throws no light on the class of animal to which the blood belongs; it may be hot-blooded or cold-blooded, a mammal or a reptile.”<sup>2</sup> . . . . . “So again the process does not enable the examiner to detect the blood-globules, or cells, or even to speak of their presence.”

This is certainly an underestimation of its value, for with proper pre-

<sup>1</sup> Vide Martinsburg “Independent,” Nov. 22, 1873.

<sup>2</sup> Guy's Hosp. Reports, vol. xiii. p. 440, 3d series, 1867.



cautions the blue-coloured disks or blood-globules can be obtained with this process; the examiner is thereby enabled to solve the question whether the blood is human or from a bird, fish, or reptile; but it affords him no light upon the question which so frequently arises, namely, whether the blood is human or from a mammalian animal.

In the fresh condition it is a comparatively easy matter to see and manipulate, so to speak, the nucleus. By the addition of a two per cent. solution of boracic acid, the elliptical blood disk swells and acquires a circular contour;<sup>1</sup> the oval nucleus becomes more and more spheroidal and yellow, while, at the same time, it increases in size. Here and there corpuscles are met with in which the yellow central body is not round, but beset with processes which stretch like rays towards the periphery; this is especially the case when a tannic acid solution of the same strength is employed;<sup>2</sup> the nuclei are apt to assume, also, a more eccentric position and after a sufficient length of time, the observer is sure to see many corpuscles in which they lie, some partly, some entirely outside of the outline of the pale disk. None of these phenomena are observed when dried blood is acted upon by these agents. Acetic acid<sup>3</sup> gives a beautiful red (hæmatine) tint to the fresh nucleus. A neutral solution of carmine,<sup>4</sup> as well as aniline colours and other substances stain, more or less intensely, the disks and their nuclei, but none of them are quite as satisfactory as that produced by the guaiacum process. It may here be observed that ether, since it is employed in obtaining this blue colouration, has the effect upon amphibian<sup>5</sup> and like blood of somewhat diminishing the size of the corpuscle, but, on the other hand, the *thickness of the margins has increased* and the *nuclei* are very clearly and *sharply defined*.

Another reason, besides its superiority, for employing the guaiacum resin and the ozonic ether in order to colour the nucleus, lies in the fact that the examiner is not dealing with substances involving the use of additional strange technical terms, but with such that he has already thoroughly familiarized his audience with. For it is not only necessary in medico-legal cases that he *himself* be satisfied that he is dealing with blood, but he ought to be able to convince all that the matter under consideration is nothing else than blood. This can readily be accomplished, even with a very small quantity of blood, by the exhibition of the oxidation of the guaiacum upon a white porcelain surface, or paper; by the production of hæmatin crystals, which also can again most readily be proved to be such by the same bluing process; finally, it should be employed under the microscope to show the presence or absence of the nucleus.

<sup>1</sup> Dr. Kline, l. c. p. 30.

<sup>2</sup> Boettcher, Virchow's Archiv, Bd. 36, p. 342.

<sup>3</sup> Henle, Allgemeine Anatomie, p. 431.

<sup>4</sup> A. Rollett, Stricker's Gewebe Lehre, p. 293.

<sup>5</sup> A. Rollett, Stricker's Gewebe Lehre, p. 291.



